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Influenza Virus-Specific RNA and Protein Syntheses in Cells Infected with Temperature-Sensitive Mutants Defective in the Genome Segment Encoding Nonstructural Proteins

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Virus-specific protein and RNA syntheses have been analyzed in chicken embryo fibroblast cells infected with two group IV temperature-sensitive (*ts*) mutants of influenza A (fowl plague) virus in which the *ts* lesion maps in RNA segment 8 (J. W. Almond, D. McGeoch, and R. D. Barry, Virology **92**:416–427, 1979), known to code for two nonstructural proteins, NS_1 and NS_2. Both mutants induced the synthesis of similar amounts of all the early virus-specific proteins (P_1, P_2, P_3, NP, and NS_1) at temperatures that were either permissive (34°C) or nonpermissive (40.5°C) for replication. However, the synthesis of M protein, which normally accumulates late in infection, was greatly reduced in *ts* mutant-infected cells at 40.5°C compared to 34°C. The NS_2 protein was not detected at either temperature in cells infected with one mutant (mN3), and was detected only at the permissive temperature in cells infected with mutant *ts*47. There was no overall reduction in polyadenylated (A^+) complementary RNA, which functions as mRNA, in cells infected with these mutants at 40.5°C compared to 34°C, nor was there any evidence of selective accumulation of this type of RNA within the nucleus at the nonpermissive temperature. No significant differences in *ts* mutant virion RNA transcriptase activity were detected by assays in vitro at 31 and 40.5°C compared to wild-type virus. Virus-specific non-polyadenylated (A−) complementary RNA, which is believed to act as the template for new virion RNA production, accumulated normally in cells at both 34 and 40.5°C, but at 40.5°C accumulation of new virion RNA was reduced by greater than 90% when compared to accumulation at 34°C.

The influenza virus genome consists of eight segments of negative-stranded RNA (4) which contain the information for seven structural polypeptides and two nonstructural polypeptides, NS_1 and NS_2 (7, 11–14, 17–19, 33).

At present, little is known concerning the functions of the nonstructural polypeptides in the virus replication cycle. NS_1 (molecular weight 23,000) is synthesized early in infection (33) and migrates into the host cell nucleus (15, 16, 19, 36), although very late in the replication cycle this protein accumulates in the cytoplasm in the form of electron-dense inclusions (26, 31). NS_2 (molecular weight 11,000) is synthesized only late in infection and remains in the cytoplasm (18, 20a). Both proteins are encoded by the smallest genome RNA segment (11, 17).

Several groups of workers have isolated temperature-sensitive (*ts*) mutants of the influenza viruses and have used these mutants to elucidate the functions of the virus structural polypeptides (20, 23, 27–30, 32, 34, 35, 38). Recently two *ts* mutants with a lesion in RNA segment 8 were isolated and characterized genetically (1, 2; J. W. Almond, Ph.D. thesis, University of Cambridge, Cambridge, England, 1977). We have begun to investigate the phenotype of these mutants by a study of the synthesis of virus-specific RNA and proteins in mutant virus-infected cells. Three types of virus-specific RNA are normally induced in influenza virus-infected cells (4, 9, 10, 37). One type (polyadenylated [A(+)] complementary RNA[cRNA]) is capped and polyadenylated, and consists of incomplete transcripts of the genome RNAs which function as mRNA's during replication. A second type [A(−) cRNA] consists of full-length non-polyadenylated transcripts of the genome RNAs which are presumed to be the templates for RNA replication. The third type consists of non-polyadenylated progeny virion RNAs (vRNA's). Our results indicate that at the nonpermissive temperature, both mutants induce the synthesis of the two types of cRNA as well as early virus-

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specific polypeptides, but are defective in late virus-specific protein synthesis and fail to induce the synthesis of new vRNA's.

MATERIALS AND METHODS

Viruses and cells. Propagation and assay of the wild-type virus were as described previously (5). The two group IV *ts* mutants, mN3 and *ts*47 (2), were propagated in fertile hen's eggs which were incubated for 42 h at 34°C. The viruses were plaque-purified before use, and the temperature sensitivity was checked by plaquing at the permissive (34°C) and nonpermissive (40.5°C) temperatures on primary chicken embryo fibroblast (CEF) cells as previously described (2).

Infection of cells. Confluent CEF monolayers were equilibrated for 1 h at either 34 or 40.5°C. Infections at 40.5°C were maintained at this temperature by immersing the bottles of cells in a Grant SP3 water bath. The medium was removed, and the cells were washed with prewarmed phosphate-buffered saline and overlaid with virus suspension in allantoic fluid equilibrated to the appropriate temperature and diluted so as to provide approximately 3 PFU/cell. Adsorption was for 30 min at 34 or 40.5°C, after which the virus was removed, and the cells were washed with phosphate-buffered saline and overlaid with prewarmed maintenance medium (199 plus 2% calf serum).

Infected-cell RNA and proteins. Infected-cell RNA was prepared, and the virus-specific RNA content was determined, as described previously (3). Synthesis of infected-cell polypeptides was examined by labeling with [^{35}S]methionine and electrophoresis on 15% polyacrylamide gels as described by Inglis et al. (12).

Virus purification. Virus strains, grown in the allantoic sacs of fertile hen's eggs as described above, were purified as previously described (12). The final virus pellet was suspended in 0.1 M NaCl–0.01 M Tris-hydrochloride (pH 7.4)–50% glycerol and stored at −20°C.

Assay of influenza virus RNA-dependent RNA polymerase. The polymerase assay procedure was based on that of McGeoch and Kitron (24). The reaction mixture contained 50 mM Tris-hydrochloride (pH 8.2), 150 mM KCl, 8 mM $MgCl_2$, 5 mM dithiothreitol, 0.5% Nonidet P-40, 2 mM ATP, 0.4 mM CTP, 0.4 mM [^{3}H]UTP, 0.2 mM GTP, 0.3 mM adenylyl (3′-5′)guanosine (ApG), and virus to a concentration of 0.5 to 1 mg of protein per ml. Reactions were carried out at either 31 or 40.5°C.

RESULTS

Temperature-shift experiments with mutants mN3 and *ts*47. Cells were infected with either mutant mN3 or *ts*47 at 34°C, and at various subsequent times the cells were shifted to 40.5°C. The virus yield at 12 h from the time of infection was measured by plaque assay (Table 1). The results show that virus growth was inhibited when cells were shifted to 40.5°C at any time up to 3 h postinfection (p.i.) in the case of *ts*47 and 4 h p.i. in the case of mN3. Shift-up at later times did not significantly inhibit virus yield (see Table 1). These results suggest that the temperature-sensitive function is required during the first 3 h (*ts*47) or 4 h (mN3) after infection.

Protein synthesis in infected cells. CEF cells were infected with the group IV *ts* mutants mN3 or *ts*47 (1, 2; Almond, Ph.D. thesis) at both the permissive (34°C) and nonpermissive temperature (40.5°C) and pulse-labeled for 30 min with [^{35}S]methionine at 2, 4, and 6 h p.i. At 34°C the normal switch from an early (2 h) to a late (4 h) pattern of protein synthesis characteristic of wild-type infections (12) was seen with both mutants. A late protein pattern is characterized by the appearance of large amounts of hemagglutinin (HA) and matrix (M) proteins (mN3, compare tracks 4, 5, and 6, Fig. 1a; *ts*47, cf. tracks 5, 6, and 7, Fig. 1b). At 40.5°C the amount of M protein synthesis never exceeded that of NS_1 protein, and protein synthesis was held at a stage corresponding to early secondary transcription (13) even as late as 6 h p.i. (mN3, cf. tracks 1, 2, and 3 with tracks 4, 5, and 6, Fig. 1a; *ts*47, cf. tracks 2, 3, and 4 with tracks 5, 6, and 7, Fig. 1b). NS_2 protein was only detected at the permissive temperature in cells infected with *ts*47 (Fig. 1b, track 7). The identity of the NS_2 protein induced by mutant *ts*47 was confirmed by comparison of its migration with authentic NS_2 protein synthesized by in vitro translation of mRNA in a wheat germ cell-free system (11). In cells infected with mutant mN3, no protein migrating in the posi-

TABLE 1. *Effect of temperature shift-up on virus yield from cells infected with group IV influenza virus mutants*[a]

Mutant	Time of shift to 40.5°C:							
	0 h	1 h	1.5 h	2.5 h	3.0 h	4.0 h	6.0 h	Not shifted
mN3	2.5×10^4	4.0×10^4	7.7×10^4	4.4×10^4	5.4×10^4	3.6×10^4	1.3×10^6	9.1×10^6
*ts*47	4.2×10^3	2.0×10^3	1.1×10^4	1.9×10^4	1.9×10^4	6.9×10^5	1.3×10^7	3.9×10^7

[a] The virus yield in the medium 12 h after infection was determined by plaque assay at 34°C on CEF cells as described previously (5).

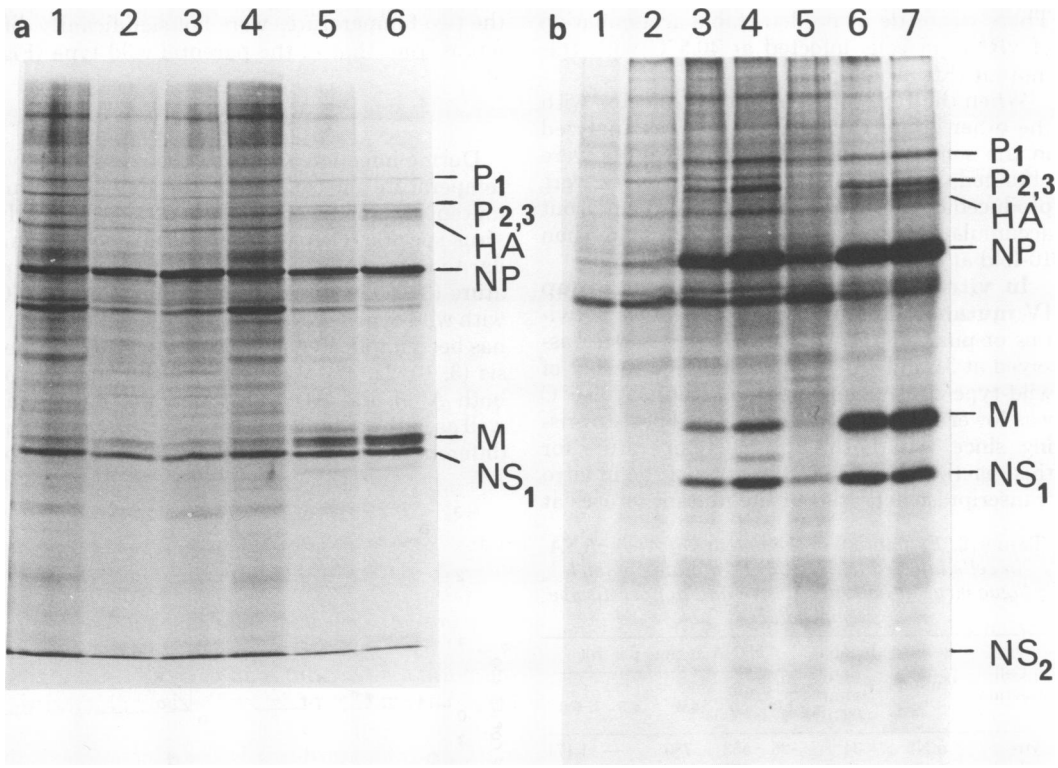

FIG. 1. *Synthesis of polypeptides in group IV mutant virus-infected CEF cells. Cells were labeled with [^{35}S]methionine for 30 min at various times after infection, immediately before harvesting. Whole-cell lysates were subjected to polyacrylamide gel electrophoresis and processed for autoradiography as described by Inglis et al. (12). Equal fractions of cell lysate were loaded onto each track. (a) mN3-infected cells. Tracks 1 to 3, 40.5°C, at 2, 4, and 6 h p.i., respectively; tracks 4 to 6, 34°C, at 2, 4, and 6 h p.i., respectively. (b) ts47-infected cells. Track 1, uninfected cells; tracks 2 to 4, 40.5°C, at 2, 4, and 6 h, p.i., respectively; tracks 5 to 7, 34°C, at 2, 4, and 6 h p.i., respectively.*

tion of NS_2 could be detected, even at the permissive temperature, despite repeated attempts using long periods of exposure to film. If the protein is synthesized in response to mN3 infection, it cannot be detected using our labeling conditions.

Accumulation of virus-specific polyadenylated RNA in infected cells. The total amounts of virus-specific A(+) cRNA were measured in cells infected with the two group IV mutant viruses at both 34 and 40.5°C (Table 2). Both virus mutants synthesized A(+) cRNA at the nonpermissive temperature. In each case, from 2.5 h after infection, the amount of virus-specific A(+) cRNA in the cytoplasm was higher at 40.5°C than at 34°C. In the nucleus the amount of A(+) cRNA was similar at both temperatures at all times analyzed (Table 2).

Accumulation of vRNA and A(−) cRNA in infected cells. The virus-specific RNA present in the A(−) fraction of RNA extracted from cells infected with mN3 at 34 and 40.5°C was examined by hybridization to ^{3}H-labeled complementary DNA and [^{125}I]vRNA as described previously (3). The accumulation of vRNA and of A(−) cRNA in infected cell nuclei and cytoplasm at both 34 and 40.5°C is shown in Fig. 2. The pattern of RNA accumulation during infection with mN3 at 34°C was similar to that found with wild-type virus (3) in that accumulation of A(−) cRNA occurred before that of vRNA. Significant amounts of A(−) cRNA and of vRNA were found in both nucleus and cytoplasm, although there was a decline in the amount of vRNA found in the nucleus at 6 h p.i.

The amount of A(−) cRNA present in the cytoplasm was greater at 40.5°C than at 34°C up to 4 h p.i. (Fig. 2b), although the amount present in the nucleus was less (Fig. 2a). From 2.5 h p.i. at 40.5°C there was little further accumulation of cRNA, any increase being associated with the nucleus rather than the cytoplasm.

There was little or no detectable accumulation of vRNA in cells infected at 40.5°C with this mutant (Fig. 2c and d).

When the RNA content of cells infected with the other group IV mutant, *ts*47, was analyzed in the same way, broadly similar results were obtained, in that A(+) and A(−) cRNA's were produced in approximately normal amounts, but accumulation of vRNA was reduced more than 10-fold at 40.5°C (Table 3).

In vitro transcriptase activity of group IV mutants. The in vitro transcriptase activities of purified *ts*47 and mN3 viruses were assayed at 31 and 40.5°C and compared to that of wild-type virus. In all cases the activity at 40.5°C was lower than that at 31°C; this is not surprising, since 31°C is the optimal temperature for this reaction. However, the ratios of the in vitro transcriptase activities of the mutant viruses at the two temperatures were not significantly different from that of the parental wild type (Fig. 3).

TABLE 2. *Estimation of the amount of A(+) cRNA in cells infected with group IV mutants of fowl plague virus at both permissive and nonpermissive temperatures*[a]

Cell fraction	Mutant	Incubation temp (°C)	cRNA at time p.i. (h): 1.0	2.5	4.0	4.5	6.0
Cytoplasm	mN3	34	26	553	780	—	1,174
	*ts*47		—	390	—	1,417	—
	mN3	40.5	32	—	1,616	—	2,274
	*ts*47		—	441	—	1,735	—
Nucleus	mN3	34	8	256	498	—	482
	*ts*47		—	156	—	397	—
	mN3	40.5	34	283	597	—	329
	*ts*47		—	55	—	294	—

[a] The polyadenylated RNA fraction was isolated from cells infected with group IV mutants of fowl plague virus both at the permissive (34°C) and nonpermissive (40.5°C) temperatures. The amount of virus-specific RNA was determined by hybridization to ^{125}I-labeled fowl plague virus vRNA as described by Barrett et al. (3). Values are expressed as nanograms of virus-specific A(+) cRNA per microgram of total RNA in the extracted cell fraction. —, Not tested.

DISCUSSION

During infection of cells at the nonpermissive temperature (40.5°C), both the influenza virus mutants studied were capable of inducing early virus-specific RNA and proteins in amounts similar to those detected at the permissive temperature (34°C) or during normal infection at 37°C with wild-type virus (3, 12, 13). Since the nucleus has been implicated as the site of cRNA synthesis (3, 10, 22, 37), we measured the amounts of both A(+) and A(−) cRNA in the nucleus as well as in the cytoplasm. However, no significant differences were observed in the amounts of

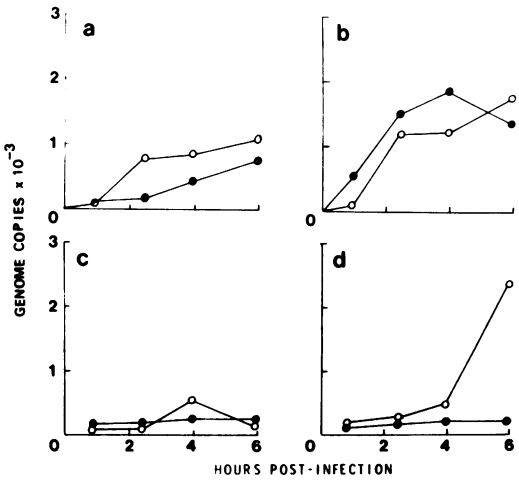

FIG. 2. *Estimation of the number of genome copies of A(−) cRNA and vRNA in the non-polyadenylated RNA fraction from the nucleus and cytoplasm of mN3 virus-infected cells.* (○) *34°C;* (●) *40.5°C. (a) A(−) cRNA nucleus; (b) A(−) cRNA cytoplasm; (c) vRNA nucleus; (d) vRNA cytoplasm. Amounts of A(−) cRNA and vRNA were determined as described by Barrett et al. (3).*

TABLE 3. *Accumulation of non-polyadenylated virus-specific RNA in CEF cells infected with ts47*[a]

Temp (°C)	Time p.i. (h)	Genome copies per cell fraction: Nucleus A(−) cRNA	Nucleus vRNA	Cytoplasm A(−) cRNA	Cytoplasm vRNA	Total A(−) cRNA	Total vRNA
34	2.5	1,090	540	1,250	530	2,340	1,070
40.5	2.5	590	350	1,250	310	1,840	660
34	4.5	460	330	3,870	5,280	4,330	5,610
40.5	4.5	370	200	720	160	1,090	360

[a] The non-polyadenylated RNA fraction was isolated from cells infected with group IV mutants of fowl plague virus both at the permissive (34°C) and nonpermissive (40.5°C) temperatures. The amount of virus-specific RNA of both positive and negative polarities was determined by hybridization to ^{125}I-labeled vRNA and a ^{3}H-labeled complementary DNA copy of vRNA as described by Barrett et al. (3).

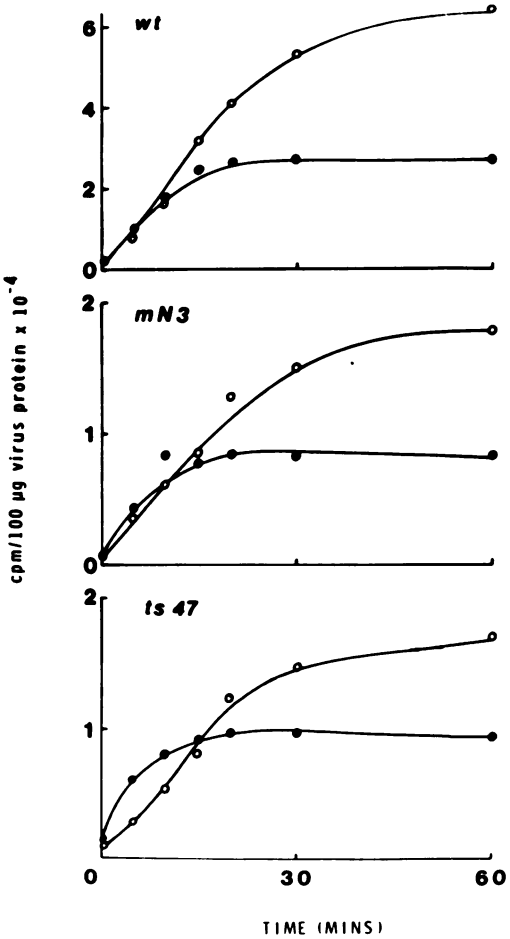

FIG. 3. *Time course of [^{3}H]UTP incorporation into RNA by purified wild-type virus and group IV mutant viruses at 31 and 40.5°C. The assay was based on that of McGeoch and Kitron (24). Each reaction contained 50 mM Tris-hydrochloride (pH 8.2), 150 mM KCl, 8 mM $MgCl_2$, 5 mM dithiothreitol, 0.5% Nonidet P-40, 2 mM ATP, 0.4 mM CTP, 0.4 mM GTP, 0.4 mM [^{3}H]UTP, 0.3 mM ApG, and virus to a concentration of 0.5 to 1 mg of protein per ml.* (○) *31°C;* (●) *40.5°C.*

A(+) cRNA present in nuclei of *ts* mutant-infected cells at 40.5°C compared to 34°C. Since the amounts of A(+) cRNA detected in the cytoplasmic fraction were higher at 40.5°C than at 34°C, there does not appear to be any block to transport of A(+) cRNA from nucleus to cytoplasm with these mutants.

We have previously shown that accumulation of new vRNA in influenza virus-infected cells begins at about 2 h p.i. (3). No such accumulation was detectable in the *ts* mutant virus-infected cells at the nonpermissive temperature. Accumulation of non-polyadenylated cRNA, which is mainly an early event in replication, occurred with both mutants at the nonpermissive temperature (3, 9). These results demonstrate that normal amounts of A(−) cRNA can be synthesized in the virtual absence of new vRNA synthesis, and so it is probable that the principal template for A(−) cRNA synthesis is input (infecting) vRNA. Since template cRNA is present in mutant virus-infected cells at the nonpermissive temperature, it is possible that one or both nonstructural proteins could play an essential role in the replicase complex. However, in addition to the block to vRNA synthesis, infection with these mutants at the nonpermissive temperature was characterized by the absence of the third (late) phase of virus-specific protein synthesis (13). The ratio of M to NS_1 polypeptides was greatly reduced at 40.5°C compared to 34°C in mutant virus-infected cells. We have previously found that during infection with wild-type influenza virus the switch to late protein synthesis occurs normally, though somewhat earlier, at 40.5°C compared to 34 or 37°C (unpublished data). This suggests that one or both NS proteins may play a role in the control of virus RNA transcription. If so, one of the late virus proteins (possibly M), rather than the nonstructural protein(s), may be directly involved in the switch from transcription to replication. Consistent with this possibility, shift-up to the nonpermissive temperature at 3 h p.i. (*ts*47) or 4 h p.i. (mN3), when normal amounts of late proteins were being produced, did not significantly affect the final virus yield. We have found a similar block to vRNA synthesis in a number of different experimental situations in which influenza virus late protein synthesis is inhibited (D. J. Briedis and B. W. J. Mahy, unpublished data).

A block to late virus-specific protein synthesis has, for example, been found in infection of cells in the presence of DNA function inhibitors such as actinomycin D or UV light (13, 21, 25) and in nonpermissive infections (6, 20a, 39). In the case of actinomycin D, there is evidence for a block in the transport of virus-specific A(+) cRNA from nucleus to cytoplasm (3, 22, 37), and accumulation of virus ribonucleoprotein has been found in nonpermissive infection (8). The intracellular distribution of virus-specific RNAs in cells infected with group IV mutants at 40.5°C gave no evidence for a similar hold-up of RNA in the nucleus.

The transcriptase activities of the group IV mutants were measured at 31 and 40.5°C to determine whether the virion polymerase was temperature sensitive in vitro, since Lamb et al. (18) reported that the NS_2 protein is associated

with the virus particle. Using the assay described by McGeoch and Kitron (24), we could detect no difference in temperature sensitivities with either mutant compared to wild-type virus under the conditions used.

Since both the influenza virus-induced nonstructural proteins are encoded in RNA segment 8 (11, 17), and the two genes may overlap, the temperature sensitivity of group IV mutants may reflect a defect in either of the proteins NS_1 or NS_2 or both. NS_1 (molecular weight 23,000) is synthesized early in infection (12, 33) and has been found associated with the nucleus (15, 19) and the nucleolus (16, 36). NS_2, on the other hand, is synthesized late in infection and is entirely cytoplasmic in location (18; 20a). Mutant *ts*47 induces the synthesis, at either permissive or nonpermissive temperature, of an NS_1 protein of reduced molecular weight (approximately 20,000) and induces the synthesis of NS_2 protein at 34°C but not at 40.5°C. Mutant mN3, on the other hand, induces the synthesis of NS_1 protein of normal molecular weight at either temperature, but the synthesis of NS_2 was not detected in our experiments even at 34°C. Since NS_2 protein is synthesized only late in normal infections, the fact that it cannot be detected with either mutant during infection at the nonpermissive temperature may merely reflect a general block to late protein and vRNA synthesis caused by a functional defect in the NS_1 protein. At present the available evidence is insufficient to decide which is the defective gene product in the mutants we have studied.

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LITERATURE CITED

1. **Almond, J. W., D. McGeoch, and R. D. Barry.** 1977. Method for assigning temperature-sensitive mutations of influenza virus to individual segments of the genome. Virology **81**:62-73.
2. **Almond, J. W., D. McGeoch, and R. D. Barry.** 1979. Temperature-sensitive mutants of fowl plague virus. Isolation and genetic characterization. Virology **92**:416-427.
3. **Barrett, T., A. J. Wolstenholme, and B. W. J. Mahy.** 1979. Transcription and replication of influenza virus RNA. Virology **98**:211-225.
4. **Barry, R. D., and B. W. J. Mahy.** 1979. The influenza virus genome and its replication. Br. Med. Bull. **35**:39-46.
5. **Borland, R., and B. W. J. Mahy.** 1968. Deoxyribonucleic acid-dependent ribonucleic acid polymerase in cells infected with influenza virus. J. Virol. **2**:33-39.
6. **Bosch, F. X., A. J. Hay, and J. J. Skehel.** 1978. RNA and protein synthesis in a permissive and an abortive influenza virus infection, p. 463-474. *In* B. W. J. Mahy and R. D. Barry (ed.), Negative strand viruses and the host cell. Academic Press, London.
7. **Compans, R. W., H.-D. Klenk, L. A. Caliguiri, and P. W. Choppin.** 1970. Influenza virus proteins. 1. Analysis of polypeptides of the virion and identification of spike glycoproteins. Virology **42**:880-889.
8. **Ghandhi, S. S., H. B. Bell, and D. C. Burke.** 1971. Abortive infection of L cells by fowl plague virus: comparison of RNA and protein synthesis in infected chick and L cells. J. Gen. Virol. **13**:423-432.
9. **Hay, A. J., B. Lomniczi, A. R. Bellamy, and J. J. Skehel.** 1977. Transcription of the influenza virus genome. Virology **83**:337-355.
10. **Hay, A. J., and J. J. Skehel.** 1979. Influenza virus transcription. Br. Med. Bull. **35**:47-50.
11. **Inglis, S. C., T. Barrett, C. M. Brown, and J. W. Almond.** 1979. The smallest genome RNA segment of influenza virus contains two genes that may overlap. Proc. Natl. Acad. Sci. U.S.A. **76**:3790-3794.
12. **Inglis, S. C., A. R. Carroll, R. A. Lamb, and B. W. J. Mahy.** 1976. Polypeptides specified by the influenza virus genome. 1. Evidence for eight distinct gene products specified by fowl plague virus. Virology **74**:489-503.
13. **Inglis, S. C., and B. W. J. Mahy.** 1979. Polypeptides specified by the influenza virus genome. 3. Control of synthesis in infected cells. Virology **95**:154-164.
14. **Inglis, S. C., D. J. McGeoch, and B. W. J. Mahy.** 1977. Polypeptides specified by the influenza virus genome. 2. Assignment of protein coding function to individual genome segmented by in vitro translation. Virology **78**: 522-536.
15. **Krug, R. M., and P. R. Etkind.** 1973. Cytoplasmic and nuclear virus-specific proteins in influenza virus-infected MDCK cells. Virology **56**:334-348.
16. **Krug, R. M., and R. Soeiro.** 1975. Studies on the intracellular location of influenza virus-specific proteins. Virology **64**:378-387.
17. **Lamb, R. A., and P. W. Choppin.** 1979. Segment 8 of the influenza virus genome is unique in coding for two polypeptides. Proc. Natl. Acad. Sci. U.S.A. **76**:4908-4912.
18. **Lamb, R. A., P. R. Etkind, and P. W. Choppin.** 1978. Evidence for a ninth influenza viral polypeptide. Virology **91**:60-78.
19. **Lazarowitz, S. G., R. W. Compans, and P. W. Choppin.** 1971. Influenza virus structural and non-structural proteins in infected cells and their plasma membranes. Virology **46**:830-843.
20. **Mackenzie, J. S.** 1970. Isolation of ts mutants and the construction of a preliminary genetic map for influenza virus. J. Gen. Virol. **6**:63-75.

20a. **Mahy, B. W. J., T. Barrett, D. J. Briedis, J. M. Brownson, and A. J. Wolstenholme.** 1980. Influence of the host cell on influenza virus replication. Philos. Trans. R. Soc. London Ser. B **288**:349-357.

21. **Mahy, B. W. J., A. R. Carroll, J. M. T. Brownson, and D. J. McGeoch.** 1977. Block to influenza virus replication in cells pre-irradiated with ultraviolet light. Virology **83**:150-162.
22. **Mark, G. E., J. M. Taylor, B. Broni, and R. M. Krug.** 1979. Nuclear accumulation of influenza viral RNA transcripts and the effects of cycloheximide, actinomycin D and α-amanitin. J. Virol. **29**:744-752.
23. **Markushin, S. G., and Y. Z. Ghendon.** 1973. Genetic classification and biological properties of temperature-sensitive mutants of fowl plague virus. Acta Virol. **17**: 369-376.
24. **McGeoch, D. J., and N. Kitron.** 1974. Influenza virion RNA-dependent RNA polymerase: stimulation by guanosine and related compounds. J. Virol. **15**:686-695.

25. **Minor, P. D., and N. J. Dimmock.** 1977. Selective inhibition of influenza virus protein synthesis by inhibitors of DNA function. Virology **78**:393–406.
26. **Morrongiello, M. P., and S. Dales.** 1977. Characterization of cytoplasmic inclusions formed during influenza/WSN virus infection of chick embryo fibroblast cells. Intervirology **8**:281–293.
27. **Palese, P., M. B. Ritchey, and J. L. Schulman.** 1977. P_1 and P_3 proteins of influenza virus are required for complementary RNA synthesis. J. Virol. **21**:1187–1195.
28. **Palese, P., K. Tobita, M. Ueda, and R. W. Compans.** 1974. Characterization of temperature sensitive influenza virus mutants defective in neuraminidase. Virology **61**:397–410.
29. **Scholtissek, C., and A. L. Bowles.** 1975. Isolation and characterization of temperature-sensitive mutants of fowl plague virus. Virology **67**:576–587.
30. **Scholtissek, C., E. Harms, W. Rohde, M. Orlich, and R. Rott.** 1976. Correlation between RNA fragments of fowl plague virus and their corresponding gene functions. Virology **84**:332–344.
31. **Shaw, M. W., and R. W. Compans.** 1978. Isolation and characterization of cytoplasmic inclusions from influenza A virus-infected cells. J. Virol. **25**:608–615.
32. **Simpson, R. W., and G. K. Hirst.** 1968. Temperature sensitive mutants of influenza A virus: isolation of mutants and preliminary observations on genetic recombination and complementation. Virology **35**:41–49.
33. **Skehel, J. J.** 1973. Early polypeptide synthesis in influenza virus-infected cells. Virology **56**:394–399.
34. **Sugiura, A., K. Tobita, and E. D. Kilbourne.** 1972. Isolation and preliminary characterization of temperature-sensitive mutants of influenza virus. J. Virol. **10**: 639–647.
35. **Sugiura, A., M. Ueda, K. Tobita, and C. Enomoto.** 1975. Further isolation and characterization of temperature sensitive mutants of influenza virus. Virology **65**: 363–375.
36. **Taylor, J. M., A. W. Hampson, J. E. Leyton, and D. O. White.** 1970. The polypeptides of influenza virus. IV. An analysis of nuclear accumulation. Virology **42**: 744–752.
37. **Taylor, J. M., R. Illmensee, S. Litwin, L. Herring, B. Broni, and R. M. Krug.** 1977. Use of specific radioactive probes to study transcription and replication of the influenza genome. J. Virol. **21**:530–540.
38. **Ueda, M.** 1972. Temperature sensitive mutants of influenza virus. Isolation and preliminary characterization. Arch. Gesamte Virusforsch. **39**:360–368.
39. **Valcavi, P., G. Conti, and G. C. Schito.** 1978. Macromolecular synthesis during abortive infection of KB cells by influenza virus, p. 475–481. *In* B. W. J. Mahy and R. D. Barry (ed.), Negative strand viruses and the host cell. Academic Press, London.